Near infrared spectroscopy reveals brain hypoxia and cerebrovascular dysregulation in primary biliary cholangitis

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Abstract: Primary Biliary Cholangitis (PBC) is an autoimmune cholestatic liver disease linked to symptoms including fatigue and altered mood/cognition, indicating that chronic liver inflammation associated with PBC can impact brain function. We employed near infrared spectroscopy (NIRS), a non-invasive neuroimaging technique, to determine whether PBC patients exhibit reduced cerebral oxygen saturation (StO2) and altered patterns of microvascular cerebral blood perfusion, and whether these alterations were associated with clinical phenotype. This observational case-control study was conducted at a tertiary hospital clinic (University of Calgary Liver Unit). Thirteen females with non-cirrhotic PBC, seven females with cirrhotic PBC, and eleven healthy female controls were recruited via physician referral and word of mouth, respectively. Near infrared spectroscopy was used to measure cerebral hemoglobin and oxygen saturation. A wavelet phase coherence method was used to estimate the coherent frequency coupling of temporal changes in cerebral hemodynamics. The PBC group demonstrated significantly reduced cerebral StO2 ($p = 0.01$, $d = 0.84$), indicating cerebral hypoxia, significantly increased cerebral deoxyhemoglobin (HHb) concentration ($p < 0.01$, $d = 0.86$), and significantly reduced hemodynamic coherence in the low-frequency band (0.08-0.15 Hz) for oxygenated hemoglobin (O2Hb) concentration ($p = 0.02$, $d = 0.99$) and tHb concentration ($p = 0.02$, $d = 0.50$), indicating alterations in cerebrovascular activity. Complete biochemical response to ursodexycholic acid (UDCA) therapy in early PBC patients was associated with increased cerebral tHb concentration and decreased hemodynamic coherence. Conclusion: Using NIRS, PBC patients were found to have hypoxia, increased cerebral hemoglobin concentration, and altered cerebrovascular activity, that were reversed in part in UDCA responders. In addition, symptoms and quality of life measures did not correlate with brain hypoxia or cerebrovascular dysregulation in PBC patients.

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Near infrared spectroscopy reveals brain hypoxia and cerebrovascular dysregulation in primary biliary cholangitis

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Abbreviations: PBC, primary biliary cholangitis; UDCA, ursodeoxycholic acid; MRI, magnetic resonance imaging; MS, multiple sclerosis; fMRI, functional magnetic resonance imaging; NIRS, near infrared spectroscopy; O_2Hb, oxygenated hemoglobin; HHb, deoxygenated hemoglobin; StO_2, oxygen saturation; functional near infrared spectroscopy, fNIRS; ALP, alkaline phosphatase; ALT, Alanine Aminotransferase; GGT, Gamma-glutamyl transferase; tHb, total hemoglobin; LF, low-frequency; VLF, very low-frequency; TNFα, tumor necrosis factor alpha; NVC, neurovascular coupling; CEC, cerebral endothelial cells; BBB, blood brain barrier.

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Primary Biliary Cholangitis (PBC) is an autoimmune cholestatic liver disease linked to symptoms including fatigue and altered mood/cognition, indicating that chronic liver inflammation associated with PBC can impact brain function. We employed near infrared spectroscopy (NIRS), a non-invasive neuroimaging technique, to determine whether PBC patients exhibit reduced cerebral oxygen saturation (StO_2) and altered patterns of microvascular cerebral blood perfusion, and whether these alterations were associated with clinical phenotype. This observational case-control study was conducted at a tertiary hospital clinic (University of Calgary Liver Unit). Thirteen females with non-cirrhotic PBC, seven females with cirrhotic PBC, and eleven healthy female controls were recruited via physician referral and word of mouth, respectively.

Near infrared spectroscopy was used to measure cerebral hemoglobin and oxygen saturation. A wavelet phase coherence method was used to estimate the coherent frequency coupling of temporal changes in cerebral hemodynamics. The PBC group demonstrated significantly reduced cerebral StO_2 ($p = 0.01$, $d = 0.84$), indicating cerebral hypoxia, significantly increased cerebral deoxy-hemoglobin (HHb) concentration ($p < 0.01$, $d = 0.86$), and significantly reduced hemodynamic coherence in the low-frequency band (0.08-0.15 Hz) for oxygenated hemoglobin (O_2Hb) concentration ($p = 0.02$, $d = 0.99$) and tHb concentration ($p = 0.02$, $d = 0.50$), indicating alterations in cerebrovascular activity. Complete biochemical response to ursodexycholic acid (UDCA) therapy in early PBC patients was associated with increased cerebral tHb concentration and decreased hemodynamic coherence.

Conclusion: **Using NIRS, PBC patients were found to have hypoxia, increased cerebral hemoglobin concentration, and altered cerebrovascular activity, that were reversed in part in UDCA responders. In addition, symptoms and quality of life measures did not correlate with brain hypoxia or cerebrovascular dysregulation in PBC patients.**

Primary Biliary Cholangitis (PBC) is an autoimmune cholestatic liver disease characterized by the immunological destruction of small hepatic bile ducts and chronic liver inflammation (1). PBC can progress to cirrhosis after many years; however, treatment with the bile acid ursodeoxycholic acid (UDCA) prevents disease progression in those who respond to therapy (1). Although prevalence estimates vary geographically, a recent US study reports a PBC prevalence of 4.3 per 100,000 in women and 1.1 per 100,000 in men, and similar findings have been reported in Canada (2). PBC is commonly associated with a number of behavioral symptoms including fatigue, itch, altered mood and cognitive changes, indicating that PBC can alter brain function (1, 3). However, the mechanism whereby PBC can impact brain function remains unknown. Importantly, PBC-associated symptoms often do not correlate with severity of liver disease, biochemical response to therapy, or changes in brain structure as measured using magnetic resonance imaging (MRI) based technologies (1, 4).

Therefore, an improved understanding of how brain changes occur in association with PBC, and their potential role in symptom development, is of significant importance to improve management of these patients.

Tight regulation of cerebral blood flow is critical to ensure normal brain oxygenation and function. Cerebrovascular dysfunction and hypoxia have been implicated in a range of neurodegenerative and neuroinflammatory disorders including Alzheimer's disease and multiple sclerosis (MS) (5, 6). We have shown, using functional MRI (fMRI), that PBC patients exhibit alterations in functional hyperemia (7), although it remains difficult to determine whether alterations in functional hyperemia arise solely from altered brain activity, or whether cerebrovascular dysfunction plays a role (8).

Near infrared spectroscopy (NIRS) allows for non-invasive measurement of oxygenated (O_2Hb) and deoxygenated hemoglobin (HHb) concentration in cortical brain tissue (9). Therefore, NIRS can be used to quantify cortical tissue oxygen saturation (StO_2), which reflects changing metabolic rate and perfusion (10). Furthermore, functional NIRS (fNIRS) can be used to probe brain activity in a similar fashion to fMRI, by assuming that the measured changes in blood perfusion and oxygenation are due to functional hyperemia, i.e. hemodynamic changes caused by neural activity (11). It is important, however, to note that due to the biophysical properties of light transmission in tissue, NIRS is limited to approximately 3 cm depth penetration, and is therefore unable to measure deep brain structures. Using NIRS in patients, altered patterns of brain blood perfusion and reduced brain oxygenation levels have been previously shown to correlate with fatigue (12) and impaired cognition (13, 14), linking reduced cortical perfusion to symptoms that have been reported to occur in PBC patients. Considering this link in the context of neuroinflammation associated with chronic peripheral inflammation, PBC-related alterations in cortical perfusion and oxygenation may contribute, at least in part, to the behavioral symptoms associated with PBC. Therefore, we undertook the following series of experiments using NIRS to determine whether PBC patients exhibit reduced cortical oxygen saturation and altered patterns of

microvascular cerebral blood perfusion, and whether NIRS findings of oxygen saturation and perfusion correlate with clinical disease markers, as well as symptom and quality of life scores in PBC patients.

Patients and Methods

Human Subjects. Thirteen females (median age 61 years; range 39-77 years) with non-cirrhotic (defined by serum biochemistry and Fibroscan® (15) scores) PBC and seven females with cirrhotic PBC (median age 59 years; range 49-65) were recruited from the University of Calgary Liver Unit. Eleven healthy female controls (median age 58 years; range 42-61 years) were recruited from the community via word of mouth. All patients were anti-mitochondrial antibody (AMA) positive. Written informed consent was obtained for all subjects prior to participation in the study. This study was approved by the University of Calgary human research ethics committee and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. Clinical markers of disease activity and severity (Fibroscan®, disease duration, and serum markers alkaline phosphatase (ALP), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), immunoglobulin M (IgM), and hemoglobin (Hgb)) and treatment response to UDCA were recorded at the time of study participation for all PBC patients. Complete response to UDCA therapy was defined as the normalization of serum ALP after starting UDCA treatment. See Table 1 for patient's clinical data.

Questionnaires

All PBC subjects completed a general quality of life questionnaire (PROMIS–29) (16), a disease-specific quality of life questionnaire (PBC–40) (17), a cognitive impairment test (6–CIT) (18), and an anxiety and depression questionnaire (HADS) (19), immediately prior to NIRS testing. See appendix Table 1 in the supplementary material.

Measurement of Resting-State Hemodynamic Coherence

A TechEn NIRSOptix continuous-wave functional NIRS (fNIRS) system was used to record changes in cerebral oxygenation, at a sampling rate of 25 Hz. A custom 44-channel head-cap that covers bilateral motor and prefrontal cortices was used to record fNIRS resting data

(Fig. 1). Light-emitting lasers transmit light at two discrete wavelengths (690 nm and 830 nm), while photodetectors continuously receive the light as it exits the tissue, producing a continuous recording of local changes in O_2Hb and HHb concentration in the cerebral cortex (9). Participants were seated in a darkened room and asked to breathe normally, limit movement, and maintain focus on a white fixation cross on a black monitor, while fNIRS data were continuously recorded for seven minutes.

We utilized a wavelet phase coherence method to calculate the synchrony of spontaneous hemodynamic oscillations across a range of frequency scales that arise from auto-regulatory processes as well as neurovascular coupling. This method has previously been applied in fNIRS and functional MRI studies to evaluate patterns of synchronous hemodynamic activity, sometimes referred to as functional connectivity (20, 21).

Pairwise (channel-to-channel) coherence for each subject was calculated for two frequency bands. The first frequency band consisted of low-frequency (LF: 0.08 - 0.15 Hz) oscillations often referred to as Mayer waves, which are thought to arise from the sympathetic regulation of blood pressure dynamics via modulation of vasomotor activity (22). The second frequency band consisted of very low-frequency (VLF: 0.01 - 0.08 Hz) oscillations, which are thought to be associated with neurovascular coupling in the cerebral cortex (i.e. functional connectivity) (23), but can also be affected by changes in systemic physiology such as breathing (24) and the arterial partial pressure of carbon dioxide (25). For each subject, a single global coherence value was determined by averaging all channel pair coherence values. See supplementary material for a schematic of the fNIRS analysis pipeline.

Quantification of Absolute Hemoglobin and Oxygen Saturation

An ISS Oxiplex (frequency-domain near infrared spectroscopy) was used to quantify absolute O_2Hb, HHb, and total hemoglobin (tHb; ie. O_2Hb + HHb) concentrations, as well as to calculate StO_2 (ie. O_2Hb/tHb) (26). A single probe was held over bilateral prefrontal cortices for 60 seconds per hemisphere. The probe was placed 3 cm from midline and 1 cm

above the eyebrow, to avoid the frontal sinus. All data were visually inspected and large spikes, which can be due to momentary uncoupling of the probe to the skin, were removed. Bilateral recordings were then averaged to produce one value for each parameter, per subject.

Group-level Statistical Analysis

Statistical comparison demonstrated that the cirrhotic PBC patients were similar to the non-cirrhotic PBC patients in terms of NIRS parameters. Therefore, all PBC patients were combined into one group for subsequent group analyses. The PBC patient group was compared to the control group for StO_2, hemoglobin concentration (O_2Hb, HHb, tHb), and coherence (O_2Hb, HHb, tHb). A non-parametric Wilcoxon rank-sum test (27) was used to test whether PBC patients differed from controls, for all NIRS parameters. Cohen's d, which is defined as the mean group difference divided by the pooled standard deviation of the data (28), was used to evaluate the magnitude of the effect in PBC patients compared to normal controls for all NIRS parameters. An effect size of d = 0.2 is considered 'small', whereas d = 0.5 is considered a 'medium' effect, and d = 0.8 is considered a 'large' effect (28).

Associations with Clinical Phenotype

Robust regression analysis was used to explore the relationship between NIRS markers of oxygen saturation, hemoglobin concentration, and coherence, and to investigate whether NIRS markers were associated with disease-specific and quality of life questionnaire scores. To determine whether NIRS markers were sensitive to treatment response in early PBC (as reflected by ALP normalization following UDCA treatment), the non-cirrhotic PBC patients were split into two groups, normal ALP (<145 U/L) and high ALP (>145 U/L). Wilcoxon rank-sum test and Cohen's d were used to test for group differences.

Results

Resting-state Hemodynamic Coherence

The PBC group had significantly reduced low-frequency (LF) (0.08 - 0.15 Hz) coherence for O^2Hb (Fig. 2*; Control:* 0.59 ± 0.06 *vs PBC:* 0.54 ± 0.04, mean ± SD, p = 0.02*, d* = 0.99) and tHb (Fig. 2*; Control:* 0.61 ± 0.06 *vs PBC:* 0.55 ± 0.04, mean ± SD, p = 0.02*, d* = 1.40) parameters, but not for HHb (Fig. 2*; Control:* 0.50 ± 0.06 *vs PBC:* 0.49 ± 0.01, mean ± SD, p = 0.25*, d* = 0.24). The difference between PBC group and controls for very low-frequency (VLF) (0.01 - 0.08 Hz) coherence approached, but did not reach significance (*O^2Hb - Control:* 0.61 ± 0.04 *vs PBC:* 0.59 ± 0.03, mean ± SD, p = 0.21*, d* = 0.50; HHb - *Control:* 0.56 ± 0.03 *vs PBC:* 0.55 ± 0.02, mean ± SD, p = 0.77*, d* = 0.14; tHb - *Control:* 0.62 ± 0.04 *vs PBC:* 0.60 ± 0.04, mean ± SD, p = 0.06*, d* = 0.82). Two PBC patients were excluded from this analysis due to insufficient signal quality.

Absolute Hemoglobin Concentration and Oxygen Saturation

Absolute StO_2 was found to be significantly reduced in the PBC group, compared to healthy controls (Fig. 3*; Control:* 61.60% ± 7.12 *vs PBC:* 55.32% ± 4.26, mean ± SD, p = 0.01*, d* = 0.84). The PBC group also had increased deoxyhemoglobin concentration (HHb) (Fig. 3*; Control:* 14.70 µM ± 5.19 *vs PBC:* 20.10 µM ± 5.91, mean ± SD, p < 0.01*, d* = 0.86), compared to healthy controls. PBC patients did not significantly differ from controls for oxyhemoglobin or total hemoglobin concentrations. Three PBC patients were found to have inadequate signal quality due to poor optode coupling and/or technical complications, and were excluded from this analysis.

Associations between NIRS and Clinical Phenotype

NIRS markers were not significantly associated with any of the symptom or quality of life scores. To explore whether NIRS measures were sensitive to biochemical markers of disease status and treatment response in early PBC, non-cirrhotic PBC patients were grouped into normal (indicating UDCA therapy responders) and abnormal (indicating UDCA therapy non-responders and/or treatment refusal). Patients who either did not respond to treatment *(n* = 4) or refused treatment *(n* = 1) had significantly reduced very-low frequency coherence (tHb; p = 0.02), and appeared to have increased levels of hemoglobin

concentration (n.s., $p = 0.17$), but no difference in oxygen saturation, compared to patients who responded to UCDA treatment *(n* = 8) (Fig.4). As expected, the non-responders (elevated ALP) also had elevated levels of GGT and ALT, which are markers of liver injury.

Discussion

It is well-established that peripheral immune activation via systemic administration of cytokines (*e.g.* tumor necrosis factor α [TNFα], interleukin-6, interleukin-1) or endotoxin (*e.g.* lipopolysaccharide) can induce neuroinflammation, alter brain function, and cause behavioral changes in mammals (29). These findings have led many to hypothesize that immune activation occurring within the body can have a deleterious effect on the brain, directly contributing to the development of behavioral changes commonly associated with a number of chronic immune-mediated diseases, including PBC. Although, the underlying mechanism whereby chronic immune activation in peripheral tissue compartments can induce changes in brain function, linked to disease-associated symptoms, remains poorly understood.

Adequate delivery of oxygen and glucose, needed to meet the metabolic demands of brain tissue, is critically important for proper brain health and function (11). This balance is achieved through highly sensitive mechanisms of blood flow regulation such as neurovascular coupling (NVC), cerebrovascular reactivity, and cerebral autoregulation. NVC is characterized by a robust increase in local blood flow in response to neuronal activity, referred to as functional hyperemia. NVC is primarily mediated through vasoactive signaling molecules such as arachidonic acid, and nitric oxide, as well as K^+ and pH, and relies on the intricate interplay between cerebral endothelial cells (CEC), glial cells, pericytes, vascular smooth muscle cells, and neurons (11). Functional hyperemia forms the basis of the blood oxygen level-dependent (i.e. BOLD) signal in functional MRI, and the oxygenated (O_2Hb) and deoxygenated (HHb) hemoglobin signal in fNIRS, which are commonly used as indirect measures of local brain activity. Using NIRS, reduced task-induced O_2Hb and HHb response have been observed in several disorders, including depression (30) and mild cognitive

impairment (14). Of particular interest, using NIRS, one group reported decreased frontal lobe oxygenation during a verbal fluency task in patients with ulcerative colitis as well as in patients with non-alcoholic fatty liver disease, but not in patients with Crohn's disease (31, 32). The authors attributed these findings to altered brain activity, although it is difficult to delineate altered brain activity from altered neurovascular coupling in patients with potential cerebrovascular dysfunction (8).

To gain a greater understanding of how chronic immune-mediated liver inflammation impacts the brain of patients with PBC, we applied a novel time-frequency (coherence) analysis in conjunction with quantitative measures of hemoglobin concentration and oxygen saturation. This analysis allowed us to probe patterns of hemodynamic activity at varying frequencies, in an effort to try and differentiate alterations in cerebrovascular function from alterations in neuronal activity. Further, quantification of hemoglobin concentrations and oxygen saturation provide insight into whether PBC patients have altered metabolism and/or are hypoxic.

Resting-State Hemodynamic Coherence

We found that PBC patients exhibit significantly reduced cortical hemodynamic coherence (Fig. 2), similar in magnitude to reductions we have previously reported in adults with MS (6) and in adults and children with chronic symptoms following mild traumatic brain injury (33, 34). In particular, our findings of reduced coherence were greatest for frequencies centered around the ~0.1 Hz oscillation (10 second rhythm) known as the Mayer wave, indicating altered vasomotor regulation of cerebral blood flow (22). Dysregulation of cerebral blood flow and perfusion in these patients may result from two potential mechanisms: 1) disrupted efferent sympathetic nervous control of arterial blood pressure, and/or 2) direct damage or injury to the vascular endothelial cells, both of which may cause dysfunctional vasomotor activity. Previously, we have shown that following bile duct ligation in mice, liver inflammation induces an immunological assault on the endothelial cells of cerebral blood

vessels (3), providing evidence of the latter as a potential driver of altered cerebral vasomotor regulation of cerebral blood flow found in patients with chronic liver inflammation.

Healthy CECs are critically important for blood flow regulation, as well as the normal function of the blood-brain barrier (BBB). Cytokines (e.g. TNFα, interleukin-1, interleukin-6) originating from within the central nervous system, or present in the peripheral blood circulation, can induce CEC dysfunction and increase BBB permeability, which is known to be associated with many neurological disorders including Alzheimer's (35), and MS (36), as well as peripherally induced neuroinflammation (3). Indeed, CECs exposed to the cytokines TNFα or interleukin-6 express fewer inter-endothelial proteins and have greater reactive oxygen species generation leading to increased BBB permeability (37). Furthermore, CECs can be activated by cytokines that are either present in the blood or released by circulating immune cells in intimate contact with CECs, to produce secondary signaling molecules (e.g. additional cytokines, prostaglandin E2, nitric oxide) which interact with cells within the brain to alter NVC responses (38, 39). Consistent with this suggestion, in a mouse model of cholestatic liver disease we have shown that activated monocytes within the cerebral circulation adhere to CECs and induce CEC activation via TNFα - TNF receptor-1 interactions (40, 41). This immune cell and TNFα driven CEC activation upregulates CEC inducible nitric oxide synthase expression and is directly linked to activation of microglia within the brain (especially those cells in close proximity to blood vessels) (40), and ultimately to altered behaviors expressed by cholestatic mice (42, 43). These previous findings from our group provide a mechanism by which peripheral inflammation can impact the function of CECs, thereby altering cerebrovascular function.

The existence of endothelial signaling pathways to neurons and astrocytes have increasingly been recognized as key regulators of NVC within the brain (11). In MS patients, similar to our findings in cholestatic mice, blocking activated immune cells within the circulation from adhering to CECs increases task-induced cortical blood flow (ie. NVC) (44), suggesting that immune cell - CEC adhesive interactions lead to neurovascular uncoupling. CECs can also

play an essential role in the regulation of cerebral blood flow by passively allowing vessel smooth muscle to relax and contract. Interestingly, activated leukocytes have been shown to promote endothelial-dependent vasospasticity and reduce arterial relaxation *in vitro* and *in vivo* (38). Further, endothelial-dependent vasospasticity is heightened in the presence of protein aggregates in the blood (38), which would effectively reduce the dynamic range of vasodilation. This is particularly interesting considering our previous findings that cholestatic liver injury leads to increased CEC presentation of the adhesion molecule P-selectin, which in turn promotes leukocyte and platelet adhesion to CECs (42, 43). Taken together, these findings suggest that increased leukocyte-CEC interactions associated with peripheral inflammation may significantly contribute to altered cerebrovascular dynamics and potential neurovascular uncoupling in PBC patients.

The second potential contributor to altered cerebrovascular activity in these patients is dysfunctional sympathetic control of the vasomotor activity that generates Mayer wave oscillations (~0.1 Hz). Under normal conditions in healthy individuals, arterial pressure is highly correlated with sympathetic nervous activity at the ~0.1 Hz frequency, indicating that autonomic control of vasodilation and vasoconstriction is essential for generating the Mayer wave (~0.1 Hz oscillation) (22). Using various methods (i.e. measuring physiological responses such as heart rate, blood pressure, and blood volume to physiological stressors such as the Valsalva maneuver, deep breathing, and sitting-to-standing) autonomic dysfunction in PBC patients has been reported by several groups, perhaps most notably in a study by Newton and colleagues (45). Interestingly, the authors found autonomic dysfunction to be associated with both cognitive impairment and frontal cortex legion load on structural MRI images of PBC patients (45). This finding links autonomic dysfunction in PBC patients to altered brain structure and function in the frontal brain region of PBC patients, supporting our observations of altered cerebrovascular activity and hypoxia in these same brain regions.

Some evidence suggests that reduced cerebral oxygenation and vasomotor activity may occur in the context of healthy aging (47, 48). In our study, all three groups had comparable mean and median age, however the PBC cohort had a wider age-range than the control and cirrhotic groups. One PBC patient in particular was well above the age distribution of the controls (Patient ID 002; 77 years of age). This patient was unremarkable in terms of hemodynamic coherence and cerebral oxygenation, suggesting that age did not impact our findings. However further studies could address the impact of age more formally to determine any potential effect of age on cerebral hemodynamics in this population.

Cortical Oxygen Saturation and Hemoglobin Concentration

We have identified a striking reduction in cortical tissue oxygen saturation (StO_2) levels in the frontal cortex of some PBC patients at rest (Fig. 3); similar in magnitude to levels previously reported by us in the frontal cortices of patients suffering with the neuroinflammatory disease MS (6). In the setting of increased platelet-immune-CEC interactions and altered vessel dynamics discussed above, we propose that hypoxia results from a physical reduction in blood flow through the brain microvasculature, coupled with a local increase in metabolic activity (due to heightened local immune cell activity). In support of this hypothesis, we observed a significant *($p < 0.01$, $d = 0.86$)* increase in deoxygenated cerebral hemoglobin in the PBC patients, without a change in total hemoglobin, suggesting that local cerebral metabolic rate of oxygen has increased without a corresponding increase in perfusion, leading to hypoxia in these patients.

In addition, hypoxia and inflammation may in fact create a positive feedback loop, which has previously been hypothesized to play an important role in MS disease progression (46). In the proposed hypoxia-inflammation cycle, hypoxia inhibits the enzyme prolylhydroxylase allowing for the activation of nuclear factor kappa B and hypoxia inducible factor-1 alpha, and subsequently the up-regulation of pro-inflammatory cytokines and leukocyte infiltration. In addition, activation of the hypoxia inducible factor-1 alpha pathway is known to selectively disrupt CEC function and increase BBB permeability (49). In this way, the hypoxic conditions

observed in some PBC patients may create a hypoxia-inflammation cycle that contributes to cerebrovascular dysfunction and further exacerbates hypoxia.

Association with Clinical Phenotype

In this study, abnormal cortical oxygenation and coherence in PBC patients were not significantly associated with symptom or quality of life measures, which may be explained by several factors. First, as previously discussed in the American Association for the Study of Liver Diseases 2018 practice guidelines, symptoms and stage of disease are not strongly correlated in PBC patients (1), indicating that symptom questionnaires are not an accurate marker of liver disease severity. It is also important to consider the overall level of symptom burden in this particular cohort. Based on prior classifications of the PBC-40 symptom inventory (50), a majority of the PBC patients in this cohort reported mild-moderate symptom burden, and only three patients (all cirrhotic) reported severe burden in any of the symptom categories. Finally, it is also possible that the abnormal markers of oxygenation and cerebrovascular activity measured here in PBC patients preclude more overt changes in behavior and function in advanced disease.

There is, however, an apparent association between NIRS markers and biochemical markers of response to therapy in early PBC (non-cirrhotic) patients. Specifically, to determine if NIRS is sensitive to treatment response in early PBC, we explored the relationship between serum ALP levels and NIRS markers of oxygen saturation, hemoglobin concentration, and cortical coherence in non-cirrhotic PBC patients. Elevated ALP is an important biomarker for PBC diagnosis and prognosis, and normalization of ALP is considered a clinical marker of complete treatment response to UDCA. In our non-cirrhotic cohort, we found that the group of patients who did not have a normal ALP level (indicating non-response to UDCA, n = 4; or treatment refusal, n = 1) demonstrated reduced coherence for the very low frequency band. Cerebral hemoglobin concentrations appeared to be increased in those patients with abnormal ALP, although there was no significant difference between these subgroups. The one patient (ID: 011) who refused UDCA therapy was, not

surprisingly, an extreme outlier for high ALP (415 U/L). Interestingly, this patient was also an outlier for cerebral hemoglobin concentration (tHb: 69 μM, O_2Hb: 41 μM, and HHb: 28 μM) and had relatively low coherence for both frequency bands (LF = 0.53, VLF = 0.59) compared to the control distribution. These findings suggest cerebral hemoglobin concentration and altered cerebrovascular activity may be biomarkers of PBC, and that improved bile flow and decreased liver inflammation, in patients who respond to UDCA therapy, may protect against deleterious brain changes associated with PBC. However, similar to widely reported clinical experience, improvements in serum ALP levels with UDCA therapy do not appear to improve PBC-associated behavioral symptoms. Moreover, our findings of UDCA-induced normalization of brain hemoglobin concentration and low-frequency coherence support the potential of using NIRS technology in future studies to evaluate disease progression and treatment response in early PBC patients.

Conclusion

Through clinical and preclinical observations, it is increasingly apparent that there is a link between peripheral inflammation, alterations in brain function, and sickness behavior development. However, the underlying pathogenic driver(s) of these brain changes are not well understood. In this study, we report the existence of cerebrovascular dysfunction, hypoxia, and increased hemoglobin concentration in patients suffering from the chronic immune-mediated inflammatory liver disease PBC. Interestingly, these alterations were not associated with symptom or quality of life changes in these patients, however, they were associated with abnormally high serum ALP in early PBC patients, suggesting that UDCA treatment response either slows or reverses brain changes associated with PBC, and that NIRS markers are sensitive to treatment response. Our findings support the potential use of the portable and non-invasive near infrared spectroscopy device as a useful neuroimaging tool to study brain changes in patients with liver disease.

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Conflict of interest statements

MGS has the following potential conflicts of interest (i) advisory board Gilead, Novartis, Intercept, Allergan, (ii) speaker Abbott, Gilead, Intercept, (iii) research funding Intercept, Gilead, CymaBay, Genkyotex, BMS, Novartis.

The other authors have no conflicts of interest to report.

Author contributions

CD: Acquisition and analysis of the data. Primary writer of the manuscript. Contributed to the development of the concept and methodology.

VA: Assisted in data acquisition, patient recruitment, data management, and general coordination of the study.

AP: Assisted in data acquisition, analysis of the data, and producing figures. Critical review of the manuscript.

FS: Contributed to the development of the processing and analysis methods. Critical review of the manuscript.

JD: Provided the NIRS technology. Contributed to the development of the concept and methodology. Significant intellectual contributions to the analysis and interpretation of the data. Critical review of the manuscript. Provided financial support.

MS: Provided access to patients and patient data. Contributed to the development of the concept and methodology. Significant intellectual contributions to the analysis and interpretation of the data. Significant contributions to the writing of the manuscript. Critical review of the manuscript. Provided financial support.

Patient ID	Age (yrs)	UDCA Response (Yes/No)	Fibroscan ® (kPa)	ALP (U/L) (N<145)	ALT (U/L) (N<60)	GGT (U/L) (N 11- 63)	IgM (g/L) (N 0.4 - 3.0)	Disease Duration (yrs)	Hgb (g/L) (N 120-160)
001	39	Y	5.1	79	36	53	1.76	5	138
002	77	Y	4.5	93	14	18	5.10	10	135
003	59	N	14.0	166	69	311	5.51	18	148
004	62	Y	5.7	85	9	37	7.25	3	131
005	67	Y	11.4	80	41	41	3.34	15	132
006	62	N	6.3	148	71	147	2.94	5	141
007	52	Y	3.5	85	30	107	3.10	1	145
008	66	N	5.6	157	26	70	2.10	9	135
009	62	Y	5.1	97	43	135	0.70	15	122
010	41	Y	6.6	104	28	114	3.14	3	126
011	61	n/a	10.7	415	66	507	4.75	9	143
012	55	N	6.0	204	43	172	4.27	1.5	148
013	54	Y	6.6	128	40	55	2.41	3.4	139
014	57	Y	17.3	135	37	44	1.7	8	89
015	65	N	35.9	169	30	49	1.03	28	125
016	49	N	n/a	228	48	92	6.17	2	119
017	62	Y	23.8	141	41	83	n/a	10	129
018	59	n/a	33.2	118	17	23	5.04	17	101
019	62	N	33	355	50	212	2.02	4.5	129
020	58	Y	63.1	89	29	127	4.73	3	137

Table 1: Patient demographics and clinical data. Patient data was compiled from review of clinical charts. UDCA treatment response is defined as normalization of serum ALP levels after starting UDCA treatment. Cirrhotic patients are shaded grey. Cirrhosis is defined by a Fibroscan > 16.9 kPa in PBC. Serum ALP, ALT, GGT, IgM, and Hgb levels were measured by Calgary Laboratory Services. *UDCA = Ursodeoxycholic acid; ALP = Alkaline Phosphatase; ALT = Alanine Aminotransferase; GGT = Gamma-glutamyl transferase; IgM = serum Immunoglobulin M; N = normal level; Hgb = hemoglobin.*

		p value	Effect size (Cohen's *d*)
Quantification of tissue hemoglobin concentration and oxygen saturation	**O_2Hb**	0.37	0.49
	HHb	**0.00	0.86
	tHb	0.17	0.83
	StO_2	*0.01	0.84
Low-frequency coherence 0.08 – 0.15 Hz	**O_2Hb**	*0.02	0.99
	HHb	0.25	0.24
	tHb	*0.02	1.40
Very low-frequency coherence 0.01 – 0.08 Hz	**O_2Hb**	0.21	0.50
	HHb	0.77	0.14
	tHb	0.06	0.82

Table. 2. Statistical values for group comparison of NIRS markers. Statistical results of Controls vs. PBC for each NIRS parameter. A Wilcoxon Rank-Sum test was used to evaluate significance (*p*-value) between PBC and control groups, for each parameter. Cohen's *d* was used to calculate the size of the effect when comparing the PBC group to the control group. ** p < 0.05, **p < 0.01; small effect is d = 0.2, medium effect is d = 0.5, large effect is d = 0.8. StO_2 = oxygen saturation, tHb = total hemoglobin, O_2Hb = oxygenated hemoglobin, HHb = deoxygenated hemoglobin.*